

Full Length Research Paper

Mineral nitrogen affects nodulation and amino acid xylem transport in the Amazonian legume *Inga edulis* Mart.

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The characteristics of symbiotic nitrogen fixation in tropical forest tree species have received little attention but it is well established that mineral N negatively affects symbioses in crop legumes such as soybean and common bean. Nevertheless, ecophysiological mineral nitrogen dynamics in terms of nitrogen xylem transport of native Amazonian legume has been not documented so far. The objective of this study was to evaluate nodulation and amino acid xylem transport of *Inga edulis*, a tropical Amazonian perennial legume, in response to mineral N. Plant growth, nodule number, nodule dry weight and xylem sap amino acid contents were determined in nodulated *I. edulis* in response to nutrition with ammonium, nitrate or no mineral N (total dependence on N₂ fixation). In addition, free individual amino acids were quantified in the different plant tissues and in the xylem. Plant growth (total dry weight) responded significantly to both nitrate and ammonium, especially the latter. Nitrate negatively affected nodulation and total amino acid transport to the shoot while ammonium increased nodule dry weight, but not total amino acid contents of the xylem which diminished. On the other hand, mineral-N showed only discrete changes in the amino acid composition of the xylem where asparagine was the predominant form. Our results suggest that *I. edulis* responds to ammonium nutrition through greater plant and nodule growth compared to the other N sources, consistent with ammonium being the principal source of mineral N in the acid soils of the natural habitat of this species.

Key words: Tropical tree species, nitrogen fixation, glutamine, symbiosis.

INTRODUCTION

Nitrogen (N) is required for all living organisms and is the fourth most abundant essential element in the plant biomass. In spite of this, little is known about the characteristics of N uses in tropical forest species since most studies have been carried out in cooler climate ecosystems (Aidar et al., 2003). In this regard, a major problem is that N limits plant growth in terrestrial

ecosystems even though the atmosphere contains some 79% of N₂. This situation is a curious paradox because aboveground plant parts are exposed to a rich concentration of N while root systems are constantly probing the soil for N (Hartwig, 1998). Under natural conditions, the ability to fix N₂ is restricted to prokaryote organisms. These microorganisms, collectively known as rizophobia, have developed a relationship with certain plant species belonging mainly to the Fabaceae family forming N fixing nodules (Rogers et al., 2009). Symbiotic nitrogen fixation (SNF) gives legumes a competitive advantage in N-poor soils and at the same time benefits other non-legume

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Table 1. Plant and nodulation growth and total amino acids of the xylem bleeding sap of *Inga edulis* fed with different nitrogen sources (N-free, NO₃⁻ or NH₄⁺).

Treatments	Plant dry weight (g. plant ⁻¹)	Nodules dry weight (g. plant ⁻¹)	Nodule number (plant ⁻¹)	Amino acids (μmol. mL ⁻¹)
N-free	13.88 ± 1.87 c*	0.387 ± 0.13 b	170.5 ± 68 a	9.07 ± 2.63 a
NO ₃ ⁻ 5 mM	18.76 ± 1.77**b	0.137 ± 0.07 c	84.8b ± 30 c	2.92 ± 2,07 c
NH ₄ ⁺ 5 mM	22.75 ± 1.75 a	0.690 ± 0.23 a	193.1 ± 45 a	6.22 ± 1.36 b

*Differences between means followed by a different letter are statistically significant by the Turkey test ($P \leq 0.05$). **Data are given as the mean of \pm standard deviation (SD) ($n = 10$).

species through a positive contribution to the N-cycle. A shortage of mineral N is the only case in which SNF is clearly favored compared to other stresses (Hartwig, 1998). SNF of the majority of leguminous species studied so far shows sensitivity to mineral nitrogen (N) but mechanisms involved in this process are still not clearly understood (Camargos and Sodek, 2010). An adequate supply of mineral N may activate a defense system in host plants that recognizes bacteria as a pathogen thereby interfering with symbiosis formation (Kirichenko, 2001). Nitrate shows a negative effect in such interaction by interfering with infection, nodule development and nitrogen fixation. On the other hand, the impact of ammonium and urea is generally weaker than that observed for nitrate (Streeter, 1988; Bollman and Vessey, 2006). Nitrate is important for plant nutrition in the tropical forest but ammonium is often the dominant N source depending on environmental and soil conditions or land uses (Aidar et al., 2003; Erickson et al., 2001; Wick et al., 2005). Reports on the effects of ammonium on legume symbiosis are less common in the literature and symbioses involving tropical legume trees have received much less attention than herbaceous types (Aidar et al., 2003; Dan and Brix, 2009; Barron et al., 2011). In Latin America, according to local farmers, the legume genus *Inga* plays an important role for improving soil nutrition (Leblanc et al., 2005), apparently by providing N to degraded lands and ameliorating soil conditions (Franco and DeFaria, 1997; Nichols and Carpenter, 2006). *I. edulis* is one of the most economically important species of the genus and is recognized as a prominent N₂-fixer in tropical biomes of South America (Leblanc et al., 2005). Nodule nitrogenase activity is a useful parameter to estimate the amount of N introduced into the biome. Alternatively, ureides exported to the shoot via xylem can be used as a means for estimating SNF (McClure and Israel, 1979) although this technique would be restricted to only those legumes that produce ureides. More recently, specific amino acid contents of the xylem bleeding sap have been demonstrated to be useful parameters to estimate SNF capacity under abiotic stress conditions (Amarante et al., 2006). In this study, we evaluated nodulation and amino acid xylem transport of *I. edulis* in response to nitrate and ammonium as external sources of N.

MATERIAL AND METHODS

Seeds of *I. edulis* obtained from fruits harvested from trees growing in Experimental Stations of the National Institute for Research in the Amazon (INPA) were germinated in vermiculite in plastic trays under natural temperature and light conditions. After 15 days, seedlings (number of leaves: three to four; height: around 12 cm) were transferred to 3 L plastic pots containing vermiculite as substrate and inoculated with a suspension of bacteria taken from 11 Amazonia *Inga* species. Plants were grown between July and October of 2010 in a greenhouse under natural light and temperature conditions and irrigated twice a week with 100 ml of full-strength modified Hoagland and Arnon's (1950) nutrient solution modified according to the following treatments with three different N sources: N-free nutrient solution or the same solution to which sodium nitrate or ammonium sulphate were added to a final concentration of 5 mM of N (EC: 2.0 dS m⁻¹). The N treatments were initiated just before inoculation. On day 98 after planting, leaves, roots and nodules were harvested and rinsed in distilled water, and the dry weights determined (after 72 h in an oven at 70°C). Amino acids were extracted from tissues by homogenization with MCW (methanol/chloroform/water – 12/5/3 – v/v) according to the procedure described by Bielski and Turner (1966). Xylem bleeding sap was collected according to McClure and Israel (1979) and total amino acids were determined colorimetrically using leucine as standard (Yemm and Cocking, 1955). Free amino acids were analyzed by reverse phase HPLC of their α -phthalaldehyde (OPA) derivatives as described previously by Puiatti and Sodek (1999). The experiment was carried out with 10 replications using a randomized complete block design. The data were subjected to an analysis of variance and significance between means determined using the Tukey test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Mineral N supplement promoted an increase of plant dry weight in comparison to plants fed with N-free nutritive solution. In this regard, ammonium had a greater positive effect on biomass of *I. edulis* as compared to nitrate (Table 1). The outstanding effect of ammonium in promoting plant growth is consistent with the importance of this mineral in the soils of the Amazon forest (Wick et al., 2005; Davidson et al., 2007), the natural habitat of this species. The inferior performance of plants totally dependent on N₂ fixation indicates that the symbiosis was not highly efficient for supplying the plant with adequate amounts of N. Both mineral nitrogen sources affected the symbiotic interaction. Nitrate significantly reduced the number and dry weight of nodules, while ammonium

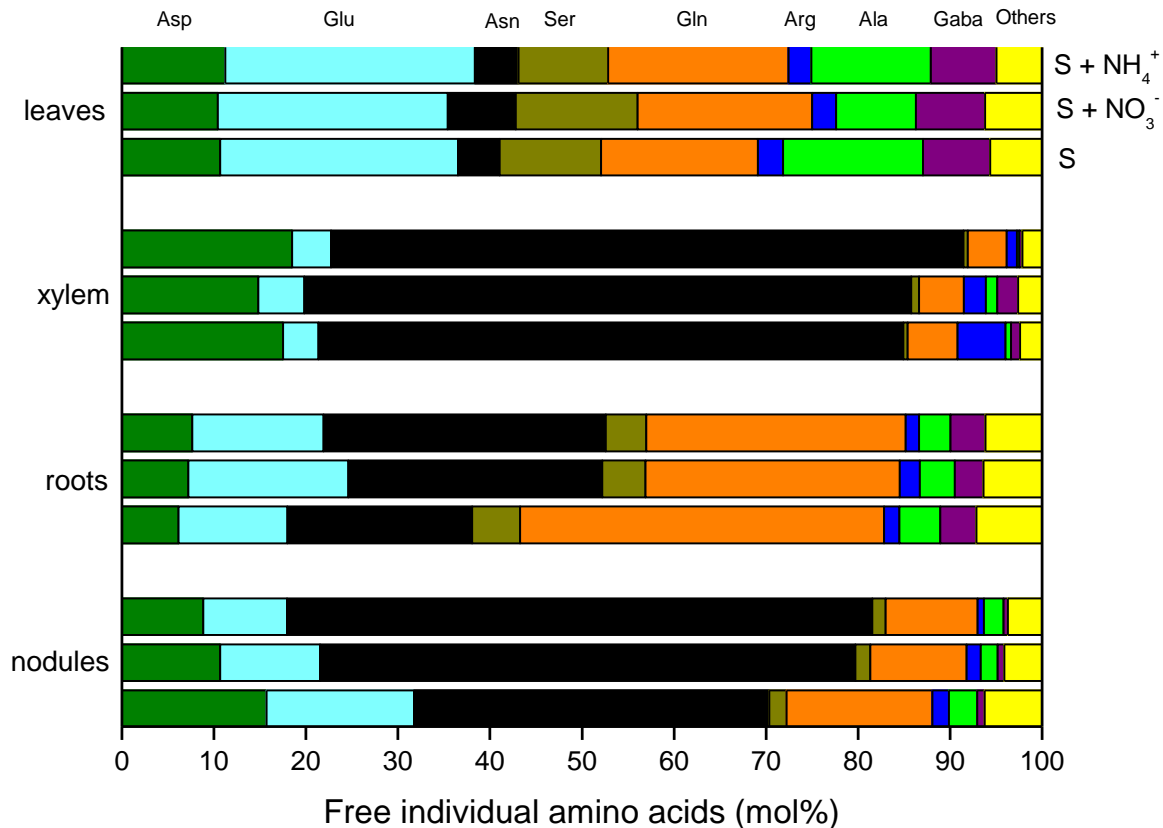


Figure 1. Amino acid composition of leaves, roots, nodules and xylem sap of symbiotic *Inga edulis* plants grown with N-free medium (S), with nitrate (S + NO₃⁻) and with ammonium (S + NH₄⁺). Symbiotic plants were cultivated during 98 days and treatment with the N source initiated three days before, and then after inoculation the symbiotic.

increased nodule dry weight (the increase in nodule number was not significant) (Table 1). Previous studies have shown that mineral N sources negatively affect nodulation (number of nodules), nodule biomass (growth of nodules) and nitrogen fixation of legumes (nitrogenase activity) (Streeter, 1988; Glyan'ko et al., 2009). According to Bollman and Vessey (2006), the negative effects of ammonium are generally less effective towards legume-rhizobium symbiosis than nitrate. However, such effects may vary in a species-specific manner. In *Sesbania sesban*, the number of nodules was more negatively affected by ammonium than by nitrate (Dan and Brix, 2009). In the case of *Calopogonium mucunoides*, a tropical South American perennial legume native to the Cerrado, neither form of mineral N (nitrate and ammonium) impaired nodule growth or nitrogenase activity (Camargos and Sodek, 2010). With respect to biomass increment, *I. edulis* responded similarly to *C. mucunoides* when plants received ammonium as N source. On the other hand, nodule formation was reduced by nitrate which is similar to results observed for most legumes studied such as soybean (Sodek and Silva, 1996). This implies that the mechanism of inhibitory influence of nitrate and ammonium on the legume-

rhizobium symbiosis is different (Glyan'ko et al., 2009) probably due the distinct N use strategies of the plants.

The amino acid composition of the roots, leaves, nodules and xylem sap was remarkably constant between treatments (Figure 1). The clearest difference in composition between treatments was found for the roots and nodules where the plants totally dependent on fixed N presented higher glutamine and lower asparagine than those given mineral N. Thus, for plants dependent on SNF in comparison with those given mineral N, glutamine was higher: 38 versus 27% for roots and 15 versus 10% for nodules, whereas asparagine was lower: 20% versus ca. 28% for roots and 38% versus ca. 60% for nodules. The largest differences in amino acid composition were found between tissues rather than treatments. Asn (38-62%) predominated in nodules, whereas in roots it was prominent (19-30%) together with glutamine (27-38%). In leaves, asparagine represented only a small fraction (<10%) of the total amino acids whereas glutamate (ca. 25%), followed by glutamine (ca.18%) was the most abundant. In the xylem sap there was little evidence for any specific amino acid being associated with either N₂ fixation or mineral N assimilation. The highest level of xylem sap amino acids (9.07 μmol.mL⁻¹) was found in

plants totally dependent on SNF, while the lowest level ($2.92 \mu\text{mol.mL}^{-1}$) was observed with nitrate (Table 1). In common with the majority of legumes (Schubert, 1986; Amarante et al., 2006; Lea et al., 2007), *I. edulis* clearly shows preference for asparagine as the main form of N transport to the shoot. Likewise, *Inga marginata* Willd grown in a secondary abandoned Atlantic Forest has been shown to transport amino acids mainly as asparagine in the xylem sap (Aidar et al., 2003). As for several non-ureide-producing legumes (Amarante et al., 2006), the amino acid composition of the xylem sap of *I. edulis* changes little in the presence of nitrate (Figure 1).

As a whole, the results indicate that the ammonium ion, in contrast to nitrate, was not inhibitory towards nodulation and nodule growth of *I. edulis*. The fact that nodule growth was enhanced by ammonium might be explained by the improved N status of the plant resulting in greater photosynthetic activity and supply of carbohydrates for plant growth. Although nitrate also promoted higher plant growth, its inhibitory effect on nodulation was apparently strong enough to more than suppress any gain in nodule growth that might result from the higher energy status of the plant. It would be interest to determine whether the superior performance of the plants grown in the presence of ammonium was in part due to a more efficient SNF, in view of the enhanced nodule growth.

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